THE CARE OF LABORATORY RATS WITH SUGGESTED NUTRITION EXPERIMENTS

Hawaii Cooperative Extension Service

Circular 396

THE CARE OF LABORATORY RATS WITH SUGGESTED NUTRITION EXPERIMENTS

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Introduction

The use of animals can be an important aid in teaching nutrition and the value of a well-balanced diet. The effects of a poor diet can be more strikingly demonstrated by animal experiments than by words and pictures. Students may learn by feeding trials which foods are rich in the necessary dietary components and how the animal's growth is affected when these components are missing from the diet. In addition to teaching students the value of a proper diet for their own health and well-being, animal experiments will encourage an interest in science and research.

White Rats as Experimental Animals

Rats are excellent animals to use in nutritional studies for a number of reasons:

1. *The rat's nutritional requirements are similar to those of man. An exception to this is vitamin C or ascorbic acid. This vitamin is necessary to the human but not to rats. Rats are able to synthesize this substance in their own bodies. Guinea pigs, like humans, are not able to make vitamin C in their bodies and it must be present in the diet.*

2. *Rats are omnivorous; they will eat almost any type of food. Dietary components can be tested with the assurance that the rats will not shun any particular food.*

3. *Rats require a relatively small amount of food, from 5 to 20 grams daily, depending upon age. Small amounts of a material may be evaluated on rats, which would be impossible using larger animals.*

4. *Rats grow fast and show differences quickly. They have a relatively short life span. The maximum age is 2½ to 3 years, which is comparable to 80 to 90 years in the human.*

5. *Rats have large litters, usually from 6 to 12. This is good for testing purposes since it is sometimes desirable to use litter-mates for different test diets.*

6. *Rats are easily and inexpensively housed and take up a small amount of space.*

Basic Biological Facts About Rats

Female rats are ready to breed at 80 days of age with maximum fertility between 100 and 300 days. The best breeding age range is from 100 to 120 days. After a rat produces 3 or 4 good litters it may become unreliable as a mother and have unhealthy young or small litters. Brother and sister matings can be made with good results.

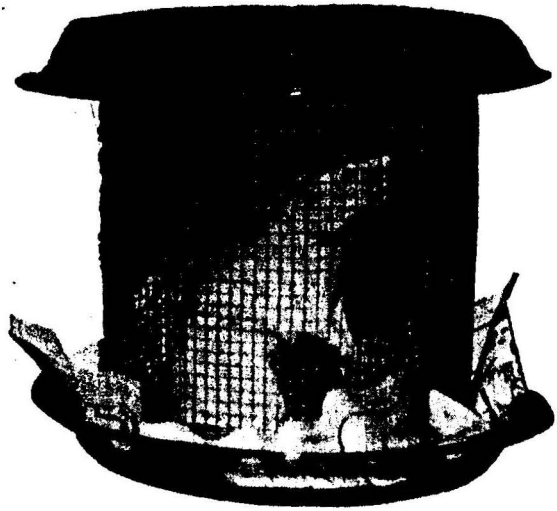
For breeding purposes, two females may be placed in a cage with one male. Pregnancy can usually be determined by a sudden gain in weight. The female rat should then be placed in a cage alone. The gestation period is 21 to 23 days. A few days before the female is expected to give birth, paper strips or shavings should be placed on the bottom of the cage. This cage should have a solid bottom so that the babies will not fall through the wire mesh. If there are more than 10 in the litter, the extra young may be adopted by another mother giving birth within a period of 2 or 3 days. If some of the babies are thin and unhealthy they should be killed. Unhealthy babies are unreliable for experimental purposes and make poor breeders. A mother should be given a rest of 2 or 3 weeks after weaning her young before she is bred again.

Baby rats weigh about 5 to 6 grams at birth. They are blind, hairless, and have closed ears. If the mother takes good care of them they grow rapidly. The ears open in 3 days and the teeth erupt at 8 days. The eyes open in 15 days. By this time the baby rats are covered with soft, white hair and are able to run and jump around. They will begin to eat solid food about the 18th to 20th day and should be weaned at 21 days. Sexes can be separated at weaning. They can be distinguished by the ano-genital distance. At 21 days the ano-genital distance is about 12 mm. in the male and an average of 7 mm. in the female. At 40 days this distance is 21 mm. in the male and 13 mm. in the female.

Rat cages should be kept very clean during the time the mother is nursing her young. The babies and mother may be placed in a clean cage daily. If another cage is not available, place the mother and litter in a box while the cage is being cleaned and fresh paper or shavings provided. The mother may have a tendency to bite if she thinks her young are being harmed, so it is best to remove her first.

Equipment Needed for Feeding Experiments

Inexpensive rat cages can be made by using wire mesh and either cake or pie pans. The wire, ¼-inch mesh, may be cut into a piece approximately 10 × 28½ inches, the exact measurements depending upon the size of the pan available. It is then rolled into a cylinder and the ends wired together. Another piece of the wire mesh cut into a circle ⅛ to ¼ inch smaller than the top diameter of the pan can be wired to the side of the cylinder for the bottom of the cage. Another pan will serve as the top of the cage. The wire cage will fit into the cake or pie pan and can be lifted easily so that the soiled paper beneath can be removed and fresh paper put in without removing the rats. (See illustration.) If lightweight pans are used, something heavy should be placed on top of the cage so that the rats cannot push the pan off and escape. Food and water cups or jars should be wired to the side of the cage so that the rat cannot turn them over.



An inexpensive cage using wire mesh and pyrex pie pans.

Routine Care of Rats

Rats should be kept in a well-ventilated room, free from drafts. They should not be subjected to extreme temperatures. The best temperature range is 75° to 85° Fahrenheit.

Rats are very susceptible to respiratory infections. If these infections are allowed to remain unchecked, pneumonia may kill a large part of a rat colony. The best means of preventing infection is by cleanliness. A rat will respond to a clean cage by keeping itself clean.

Soiled papers beneath the cage should be removed each day and replaced with clean paper.

Water cups should be washed well and fresh water given daily.

Give fresh food daily in clean cups or jars. Dry foods such as pieces of bread or vegetables may be placed on the bottom of the cage but should be removed each day.

Extra food may be given on Saturday so that it is not necessary to feed the animals on Sunday, but they should be fed early Monday morning.

Once each week move rats temporarily into another cage or box. Wash the cage, bottom and top pans, and wires with hot, soapy water. Rinse and let dry before replacing the rats.

Rats w ey are handled frequently and gently from the l. Ordinarily, they will not bite if handled pr mal around the back just behind the front legs. by the tail. It may twist around so that you may be skin of the rat's tail.

Suggestions periments

Before b dies, there are a number of things the p in mind:

1. All humane. If your experiment gives nafic investigation or makes them ential for their own well-being, can be gained by inflicti animal to become emac diet, replace it wit and symptoms become evid kill the animals by placin ith ether or chloroform.
2. Use o ible. Since animals build up vitamins and minerals, it will take about 4 w deficiency project using mature rats or and should not be attempted. Be sure to allo r experiment.
3. Since male and at different rates, either use the same sex on d experimental diets, or use a male and a female diet.
4. The experiment sh up to show definite results. Diets should be different e so that a weight change or symptoms of deficiency are me gful. Dramatic results can be achieved with 1 or 2 rats, eac extremely poor and good diets. A much larger number of a ls is necessary to show small differences in the adequacy of ts.
5. A diet containing 15 to 20 percent protein, 55 to 65 percent carbohydrate, and 5 to 10 percent fat, plus the necessary vitamins and minerals, will provide good growth and development. Plan the amount carefully to contain all necessary components.

Collecting and Presenting Data

Keeping an accurate record of weight changes and food consumption is an important part of animal dietary experiments. A card in the form shown below is a convenient way of keeping such records.

Weight Change and Food Consumption Record

Rat Weight:

Rat No.:

Diet:

Date	Food given gm.	Left-over gm.	Food eaten gm.	Rat weight gm.	Comments

The rats should be watched closely for abnormal symptoms and notations made under comments. Some symptoms to watch for are scaly tail and legs, loss of hair, diarrhea, watery eyes, unsteady gait, and nervous symptoms. It should be remembered that most symptoms are not specific. Although a vitamin-A deficiency may cause watery eyes, this condition may also be due to an eye infection which will clear up

in a few days. Students doing more advanced experiments should keep in mind that even though they plan their experiments to show definite results, the unexpected sometimes happens. This should interest rather than discourage them and they should be encouraged to discuss reasons for their results.

A written report of the experiment should include: (1) an introduction which states the purpose of the experiment with some background information; (2) experimental methods given in detail; (3) results which describe symptoms, if any, and a graphical presentation of weight gains and food consumption; (4) conclusions and discussion of results; and (5) summary.

Suggested Diets

I. Elementary experiments

	Good diet	*Poor diet*
A.	School lunch in proportions served to children with milk replacing water.	Bread or crackers with soda pop replacing water. No milk.
B.	Milk mixed with egg yolk (or other food containing iron).	Milk only.
C.	Choose 1 item from each group (A, B, and C) below.	Choose 3 items from any one group (A, B, or C) below.

GROUP A
(high protein)

meat	cheese
fish	tofu
eggs	aburage
milk	beans

GROUP B
(high carbohydrate)

bread	white potatoes
brown rice	sweet potatoes
cereal	rolled oats
taro	bananas

GROUP C
(high vitamin)

carrots	watercress
lettuce	spinach
cabbage	turnip tops
papaya	peas

II. Advanced experiments

A. *The effect of water and alcohol extraction of a diet on the growth of rats.*

Choose one item from each group above, such as cheddar cheese, brown rice, and carrots in a 20:70:10 proportion.

Cook rice in equal parts of water and cool. Grind cheese and carrots. Mix together thoroughly 70 gm. of rice, 20 gm. of cheese, and 10 gm. of carrots. Divide into three lots. To one lot, or one-third of the mixed diet add water about 3 times the volume of the diet portion. Mix thoroughly and put through 2 thicknesses of cheesecloth. Squeeze out excess liquid. Repeat this 2 or 3 times. Spread out on paper toweling and allow to dry. In the same way, extract the second one-third of the diet using ethyl alcohol and allow to dry. Use the remaining portion of the mixed diet as a control. Put 2 or 3 rats on each diet and observe the growth response.

This experiment will show that water removes certain components, while alcohol removes others. You will note that the alcohol removes much of the fat from the cheese, while the water removes starch from the rice. Note differences in the color and general appearance of the water and alcohol extracts and also of the extracted diets.

B. *The effect of prolonged cooking on the nutritive value of a diet.*
Choose a good diet. Mix your dietary components and divide the mixture into two lots. Cook one lot in excess water beyond normal cooking time. Cooking time depends upon the components selected. Foods that are normally soft and edible in 10 to 15 minutes should be cooked for 1 hour. Pour off the excess water. Feed the uncooked portion as a control. This experiment will show that certain nutrients, particularly the B vitamins, are lost by cooking in excess water.

While prolonged cooking in excess water will give the most striking results, this experiment can be varied in many ways by an ingenious pupil. You might try comparing (a) short-time cooking in a small amount of water and saving the water, and (b) short-time cooking in excess water and discarding the water. Or,

you may use canned vegetables with and without the liquid and compare the two diets. These experiments will show why nutritionists recommend cooking vegetables in a small amount of water and avoiding overcooking.

C. *The effect of exercise on the amount of food consumed.*
Build an exercise cage by using a round cage with an axle running through the center so that the cage moves around as the animal runs. Keep one rat in this cage during the day and another rat in small and cramped quarters so that it cannot move around freely. A small cage can be made with wire mesh as suggested earlier. Food, in weighed amounts, should be provided to both rats only at night. Keep a record of the weight gain and the amount of food consumed.

D. *The effect of a low-protein diet on growth.*
Using the Food Composition Table given in the Appendix, choose a diet containing 8 to 10 percent protein for the low-protein diet and 15 to 20 percent protein for the control. Be sure that both diets contain good sources of the various minerals and vitamins listed. An example of a low-protein and a control diet is as follows:

	CONTROL. *grams/100 gm. diet*	LOW PROTEIN *grams/100 gm. diet*
Tuna fish	40	10
Brown rice (cooked)	43	63
Brewer's yeast	10	10
Carrots	2	2
Mung beans	5	5

This experiment might also be set up to show the effect of a high- or low-fat diet.

E. *The effect of a milk diet on blood formation.*
Milk is low in iron and copper which are necessary for the formation of red blood cells. With milk as the only dietary component rats will become anemic. This can be shown as follows: Place two young rats on a milk diet. Either condensed or fresh milk may be used. If fresh milk is used, additional water need not be provided.

Perform hematocrit determinations (percentage of the total volume of blood due to red blood cells) twice weekly. This can be done in the following way: Using melting point capillary tubes or hematocrit tubes sold especially for this purpose, make a small cut in a tail vein of the rat and let the blood flow into the capillary tube. Seal the other end of the tube by holding it in the flame of a bunsen burner. Centrifuge the tubes for approximately 30 minutes at high speed to separate the red cells and serum. Measure with a ruler the total volume of blood from the tip of the tube to the top of the serum and also the volume of red cells.

$$\text{Hematocrit} = \frac{\text{red cell volume} \times 100}{\text{total blood volume}}$$

For normal rats the hematocrit value is from 40 to 45 percent.

When the hematocrit value has been reduced to 35 to 38 percent, supplement the milk diet of one rat with a solution containing 1 mg. of iron chloride and 0.15 mg. copper sulfate daily. Continue the hematocrit determinations. Note the color of the tail, ears, and eyes. If a microscope and counting chamber are available, counts of red blood cells may be compared with the hematocrit values.

F. *The toxicity of vitamin A.*
Vitamin A in excessive amounts is toxic to rats and also to human beings. Approximately 1,000 times the normal daily requirement of this vitamin will cause growth and skeletal changes within a few weeks. This can be shown by the following experiment:

Place two young rats on a balanced diet of your choosing. Supplement the diet of one rat with 15,000 to 25,000 I.U. (International Units) of vitamin A concentrate. The vitamin A concentrate may be diluted with liquid cooking oil and 1 to 2 drops of the mixture fed to the rat with a plastic eye dropper. Record weight changes.

Since the bones of rats that are fed excessive amounts of vitamin A become fragile and break easily, the supplementation should be discontinued after the first symptoms of weight loss and limping become apparent.

Use of the Food Composition Table

The Food Composition Table has been included in this circular to provide information on the nutritive value of some locally-produced foods as well as foods used generally The values of the vitamins, minerals, and proximate composition are given for 100 grams of the food material with an approximate measure in household units. A table such as the one given below will help in formulating a diet.

Diet Formulation Table

Food	Food in diet *gm.*	Protein *gm./100 gm*	Protein in diet *gm.*	Carbohydrate *gm./100 gm.*	Carbohydrate in diet *gm.*	Fat *gm./100 gm.*	Fat in diet *gm.*
Beef heart	50	16.9	8.95	.7	.35	3.7	1.85
Brown rice	50	7.5	3.75	77.7	38.85	1.7	.85
Total	100		12.70		39.20		2.70

The author does not recommend the use of synthetic diets in student experiments. Much more can be gained by working with the food we eat.

Food	Wt. gm.	Approximate measure	Calories	Protein gm.	Fat gm.	CHO[1] gm.	Ca[2] mg.	P[3] mg.	Fe[4] gm.	A I.U.	Vitamin Thiamine mcg.	Vitamin Niacin mg.	Vitamin Riboflavin mcg.	Vitamin C mg.
FISH AND SEA FOOD														
Fish cake, broiled	100	9 slices, ¼" thick	129	13.0	0.8	15.9	7	48	0.2	0	9	1.7	12	0
Bagoong	100	½ cup	52	11.0	6.0	0	870	427	8.6	0	0	0	0	0
Cooked shrimp	100	¾ cup	127	26.8	1.4	0	115	263	3.1	60	10	2.2	30	0
Tuna fish	100	3¼ oz., drained	198	29.0	8.2	0	8	351	1.4	80	50	12.8	120	0
MEATS														
Heart, beef, raw	100	3½ oz.	108	16.9	3.7	.7	9	203	4.6	30	580	7.8	890	6
Liver, beef, raw	100	3½ oz.	136	19.7	3.2	6.0	7	358	6.6	43900	260	13.7	3330	31
Beef, hamburger	100	3½ oz.	364	22.0	30.0	0	9	158	2.8	0	80	4.8	190	0
Pork, lean, raw	100	3½ oz.	357	14.5	32.7	0	8	157	2.2	0	70	3.8	170	0
Ham, fresh	100	3½ oz.	344	15.2	31.0	0	9	168	2.3	0	740	4.0	180	0
Veal, cutlet	100	3½ oz.	219	28.0	11.0	0	12	258	3.5	0	80	6.1	280	0
FRUITS														
Apple	100	1 small	58	.3	.4	14.9	6	10	.3	90	40	.2	30	5
Banana	100	½ cup	89	1.3	.2	23.1	4	20	.2	150	26	.6	38	6
Guava	100	1 medium	30	.2	.1	8.3	9	9	.2	200	35	.6	43	100
Papaya	100	½ small	43	.4	.1	11.6	29	11	.2	1047	25	.3	41	84
Passion fruit juice	100	½ cup	50	.4	0	13.6	4	13	.2	717	—	1.5	131	30
Pineapple, fresh	100	½ cup	41	.4	.2	10.5	14	9	.2	—	66	.2	28	8
Raisins, dry	100	½ cup	268	2.3	.5	71.2	78	129	3.3	50	150	.5	80	0
NUTS														
Coconut, grated	100	1 cup	306	2.9	29.6	11.9	18	84	1.7	0	27	.6	—	0
Walnuts	100	¾ cup	654	15.0	64.9	15.6	83	380	2.1	30	48	1.2	130	3
Macadamia nuts	100	¾ cup	727	9.2	78.2	9.9	53	240	2.0	0	340	1.0	93	0
DAIRY PRODUCTS														
Butter	100	7 teaspoons	716	.6	81.0	.4	20	16	0	3300	trace	.1	10	0
Cheese, Cheddar	100	4 x 1" cubes	398	25.0	32.2	2.1	725	495	1.0	1400	20	trace	420	0
Milk, cow	100	½ cup	68	3.5	3.9	4.9	118	93	.1	160	40	.1	170	0

Food	Wt. gm.	Approximate measure	Calories	Protein gm.	Fat gm.	CHO[1] gm.	Ca[2] mg.	P[3] mg.	Fe[4] gm.	Vitamin A I.U.	Thiamine mcg.	Niacin mg.	Riboflavin mcg.	C mg.
CARBOHYDRATE FOODS														
Rice, brown	100	½ cup	360	7.5	1.7	77.7	8	170	.8	0	408	5.0	67	0
Rice, white	100	½ cup	355	6.7	.7	77.8	9	97	.2	0	91	1.9	30	0
Sweet potatoes, boiled	100	1 medium	123	1.8	.7	27.9	30	49	.7	7700	90	.6	50	20
White potatoes, boiled	100	1 medium	83	2.0	.1	19.1	11	56	.7	20	100	1.2	40	15
Bread, white	100	6 slices	275	8.5	3.2	51.8	79	92	1.8	0	240	2.2	150	0
Bread, whole wheat	100	6 slices	240	9.3	2.6	49.0	96	263	2.2	0	300	3.0	130	0
Bread, rye	100	6 slices	244	9.1	1.2	52.4	72	147	1.6	0	180	1.5	80	0
Corn flakes	100	4 cups	385	8.1	.4	85.0	11	58	2.2	0	410	2.2	100	0
Rolled oats	100	4 cups	396	14.5	7.0	70.2	160	350	4.1	0	820	1.9	190	0
Taro	100	½ cup	104	1.0	.1	25.0	18	44	1.0	trace	111	.5	30	5
VEGETABLES														
Carrots, raw	100	1 cup	42	1.2	.3	9.3	39	37	.8	12000	60	.5	60	4
Turnips, raw	100	½ cup	32	1.1	.2	7.1	40	34	.5	trace	50	.5	70	28
Cabbage, raw	100	1 cup	24	1.4	.2	5.3	46	31	.5	80	60	.3	50	50
Watercress	100	2 cups	15	1.9	.3	2.3	88	44	.6	1197	77	.5	130	56
Turnip greens	100	16-19 plants	53	1.8	.3	13.0	53	59	—	3760	5	.1	259	0
Spinach, cooked	100	1¼ cups	26	3.1	.6	3.6	124	33	2.0	11780	80	.6	200	30
Peas, cooked	100	1⅓ cups	70	4.9	.4	12.1	22	122	1.9	720	250	2.3	140	15
Beans, green snap, cooked	100	½ cup	22	1.4	.2	4.7	36	23	.7	660	50	.4	90	10
Bamboo shoots	100	½ shoot	27	2.6	.3	5.2	13	59	.5	20	150	.6	70	4
Bean, mung, dry	100	½ cup	339	24.4	1.4	59.7	91	320	6.3	40	680	2.0	210	3
Tofu	100	¼ block	72	10.0	4.0	1.0	142	156	1.6	0	73	.2	20	0
Soybean, fresh	100	½ cup	112	12.2	2.8	11.5	82	225	2.3	582	288	1.6	190	26
Lettuce, head	100	4 large leaves	15	1.2	.2	2.9	22	25	.5	540	40	.2	80	8
MISCELLANEOUS														
Egg, whole	100	2 medium	162	12.8	11.5	.7	54	210	2.7	1140	100	.1	290	0
Yeast, brewers	100	1 cup	273	36.9	1.6	37.4	106	1893	18.2	0	9630	36.2	5450	0

[1]Carbohydrate
[2]Calcium
[3]Phosphorus
[4]Iron

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